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**ONTOGENETIC AND
PHYLOGENETIC MECHANISMS OF
NEUROIMMUNOMODULATION**

**FROM MOLECULAR BIOLOGY TO
PSYCHOSOCIAL SCIENCES**

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**Expression of the Natural Killer (NK)
Cell-Associated Antigen CD56(Leu-19),
Which Is Identical to the 140-kDa
Isoform of N-CAM, in Neural and
Skeletal Muscle Cells and Tumors
Derived Therefrom^a**

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CD56 is a newly defined cluster of leucocyte differentiation (CD) antibodies which consists of six monoclonal antibodies (mAb) including Leu-19. The corresponding CD56 antigen is a 220/140-kDa cell surface glycoprotein corresponding to the natural killer (NK) cell-associated molecule NKH-1.¹ Recently, Lanier *et al.* found CD56 also expressed in neural tissue and were able to demonstrate its identity to the 140-kDa isoform of the neural cell adhesion molecule N-CAM.²

N-CAM, an integral membrane glycoprotein and member of the immunoglobulin supergene family, is one of a group of membrane-bound cell adhesion molecules that mediates adhesion between neurons and between neurons and myotubes.³ A considerable number of molecular isoforms have been found which make up two main categories, namely, brain and muscle N-CAM.^{4,5} Furthermore, soluble forms of N-CAM have been isolated.⁶ These major isoforms are generated from a single copy gene located on chromosome 11 in humans by alternative RNA processing at the splicing and polyadenylation levels.^{7,8} They differ mainly in the amounts of sialic acid at the carboxyterminal ends of the molecules, reflecting different stages during embryogenesis. In the normal state, N-CAM is present in all three germ layers during early development.⁹ In the adult, however, it is mainly restricted to neural cells.³ The identity of CD56 with the 140-kDa isoform of N-CAM prompted us to examine its expression in a comprehensive series of normal neural and striated muscle cells and their tumors by application of monoclonal antibody (mAb) Leu-19.

EXPERIMENTAL DESIGN

Fresh tissue samples of a comprehensive series of normal and regenerative neural and skeletal muscle cells and tumors derived therefrom (see TABLES 1a,b)

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TABLE 1a. Expression of CD56 in Nonneoplastic Neural and Skeletal Muscle Cells

| Cell Type | CD56 |
|-------------------------------------|--------------------|
| Thin peripheral nerve fibers | + |
| Thick peripheral nerve fibers | - |
| Fine varicose nerve endings | + |
| Regenerative nerve fibers | + |
| Meissner's corpuscles | + |
| Pacinian corpuscles (axons) | + |
| Pacinian corpuscles (lamellae) | - |
| Intrafusal muscle fibers | +/- |
| Ganglion cells | + |
| Satellite cells | + |
| Chromaffine cells (adrenal medulla) | + |
| Fetal skeletal muscle cells | + |
| Adult skeletal muscle cells | -/(+) ^b |
| Regenerative skeletal muscle cells | +/(+) |

^a Scoring of cell reaction: +, strong staining intensity for all cells; (+), weak staining intensity for all cells; +/-, positive and negative cells in various amounts, -, negativity for all cells.

^b Weak positivity in some muscle fibers of eye muscles and of the tongue.

were snap-frozen in liquid nitrogen and stored at -70°C until use. Diagnosis of the tumors was based on standard histopathological criteria as described by Enzinger and Weiss.¹⁰ Serial frozen sections of a thickness of 4-6 µm were air-dried, acetone-fixed at room temperature for 10 min, and immediately stained or stored at -20°C for a short period. An indirect streptavidin/biotin-peroxidase method served as detection system for the immunohistochemical procedure (for details see REF. 11). MAb CD56(Leu-19, diluted 1:20 in phosphate buffered saline solution) was obtained from Becton-Dickinson (Mountain View, CA). MAb CD57(Leu-7, diluted 1:20) recognizing the myelin-associated glycoprotein (MAG) and mAb

TABLE 1b. Expression of CD56 in Tumors of Neural and Skeletal Muscle Origin

| Phenotype | Number | + | +/- | - |
|-----------------------------|--------|-----------------|-----|---|
| Benign schwannoma | 3 | 3 | - | - |
| Malignant schwannoma | 13 | 5 | 3 | 5 |
| Peripheral neuroepithelioma | 4 | - | 1 | 3 |
| Ganglioneuroma | 4 | GC ^b | 4 | - |
| | | SC ^c | 4 | - |
| Ganglioneuroblastoma | 4 | GC ^b | - | 4 |
| | | NB ^d | 4 | - |
| Neuroblastoma | 4 | 4 | - | - |
| Rhabdomyosarcoma | 8 | 6 | 2 | - |

^a Scoring of tumor cell reaction: +, strong positivity for all tumor cells, +/-, strongly and/or weakly positive and negative tumor cells in various amounts, -, negativity for all tumor cells.

^b GC = ganglion cells.

^c SC = Schwann cells.

^d NB = neuroblasts.

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NR4 directed against 68-kDa neurofilament (diluted 1 : 50), which served as structural markers in neural tissues (data not shown), were purchased from Becton-Dickinson and Dakopatts (Copenhagen, Denmark), respectively. MAb HD77 (diluted 1 : 100), directed against the pan leuco-histiocytic antigen CD53, was raised in our own laboratory (in collaboration with Dr. B. Dörken and Dr. G. Moldenhauer) and allowed a clear discrimination between interstitial dendritic cells and nonneoplastic and neoplastic neural cells, respectively (data not shown).

RESULTS

The expression of CD56 in nonneoplastic neural and skeletal muscle cells as well as in their tumors is shown in TABLES 1a,b.

In brief, CD56 was expressed in the great majority of normal neural cells of the peripheral and autonomic nervous system (FIGS. 1A,F). Only thick nerve fibers, lamellae of Pacinian corpuscles and a minority of intrafusal muscle fibers were CD56-negative. Furthermore, the majority of haphazardly oriented nerve fibers in all traumatic neuromas studied were CD56-positive (FIG. 1B). Among the tumors of the peripheral nervous system studied, all benign schwannomas were CD56-positive. However, malignant schwannomas exhibited a heterogeneous pattern of expression of CD56, *i.e.*, some tumors were consistently CD56-positive whereas others were only partially CD56-positive or even lacked these molecules completely (FIG. 1C). Likewise, peripheral neuroepitheliomas were in most cases completely CD56-negative (FIG. 1H). In contrast, the small, round, undifferentiated tumor cells of all (ganglio-)neuroblastomas studied were CD56-positive as were some of the immature ganglion cells of the ganglioneuroblastomas (FIG. 1D). Some of the ganglion cells of ganglioneuromas lacked satellite cells and showed clearly discernable CD56-positive cell membranes; mature ganglion cells in addition showed CD56-positive satellite cells. Furthermore, the majority of Schwann cells were CD56-positive in these tumors.

Fetal skeletal muscle cells of 18 weeks of gestation were strongly, those of 35 weeks were weakly CD56-positive compared to strongly CD56-positive thin nerve fibers of adjacent nerve trunks (FIG. 1E). Normal adult skeletal muscle cells were CD56-negative except for some muscle fibers of eye muscles and of the tongue (FIG. 1F). In contrast, the overwhelming majority of muscle fibers microtopographically associated with malignant and optionally even with benign tumor cells were CD56-positive as were most muscle fibers in scarred tissue (FIG. 1H). Finally, CD56 was consistently expressed in the majority of rhabdomyosarcomas studied (FIG. 1G); only a few cases showed a CD56-negative tumor cell subpopulation.

DISCUSSION

Although the distribution pattern of N-CAM has been extensively studied in vertebrates, information on its pattern of expression in human tissue is still scarce. The identity of CD56 with the 140-kDa isoform of N-CAM prompted us to study its expression in a comprehensive series of nonneoplastic neural and skeletal muscle cells and their tumors by application of mAb CD56(Leu-19).

We found the CD56 antigen expressed in the great majority of nonneoplastic cells of the peripheral and autonomic nervous system. The results on CD56 expression in thin human nerve fibers by absence in thick ones are in accordance with data

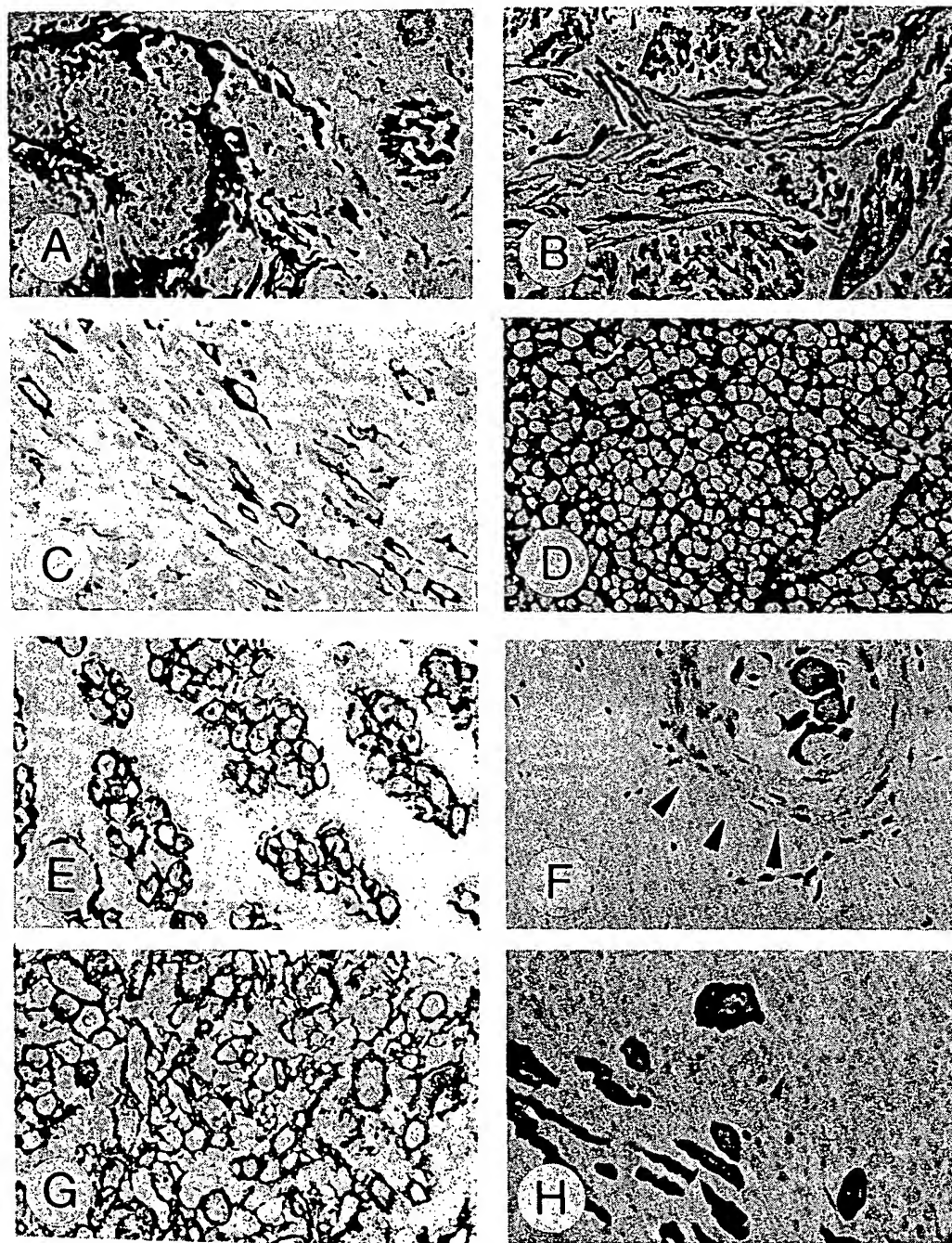


FIGURE 1. CD56 is consistently expressed in thin nerve fibers and fine varicose nerve endings (A), in haphazardly oriented nerve fibers of a traumatic neuroma (B), and in an undifferentiated neuroblastoma (D). A malignant schwannoma shows a CD56-positive tumor cell subpopulation (C). Fetal skeletal muscle fibers are strongly CD56-positive (E), whereas adult ones lack any detectable CD56 molecules (F). Some intramuscular muscle fibers, however, are CD56-positive (F, arrows). In an embryonal rhabdomyosarcoma both undifferentiated tumor cells and elongated or rounded rhabdomyoblasts display strong staining for CD56 (G). The undifferentiated tumor cells of a peripheral neuroepithelioma are CD56-negative, whereas infiltrated skeletal muscle fibers are strongly CD56-positive (H).

by Nieke and Schachner who demonstrated expression of N-CAM in unmyelinated Schwann cells in adult rats.¹² In traumatic neuromas and in the Schwann cell compartment of ganglioneuromas, CD56 was more broadly distributed and could also be observed in thick Schwann cells which showed a co-expression of CD57 (data not shown). These findings implicate parallels to the reappearance of N-CAM in the distal ends of transected rat ischiadic nerves.^{12,13} Furthermore, CD56 was broadly expressed in benign schwannomas. Malignant schwannomas, however, exhibited a heterogeneous pattern of CD56 expression, *i.e.*, some cases were only partially CD56-positive or even lacked these molecules completely. The absence of at least the 140-kDa isoform of N-CAM in some malignant schwannomas might lead to a loss of contact inhibition resulting in cell migration in an uncontrolled manner. This suggestion is supported by the finding according to which the metastatic melanoma cell line K1735-M1 expressed less N-CAM than did the nonmetastazising K1735-C116 line.¹⁴ Furthermore, N-CAM expression is markedly reduced after transformation of retinal and neural cells with Rous sarcoma virus.¹⁵ N-CAM expression in neuroblastomas was initially described in the mouse showing a shift towards the 180-kDa isoform at later stages¹⁶ and subsequently also in humans using a rabbit anti-human N-CAM antiserum.¹⁷ Using the same mAb we applied, Feickert *et al.* found CD56 expression in only 6/11 neuroblastomas,¹⁸ whereas we found CD56 expressed in all (ganglio-)neuroblastomas studied, irrespective of the differentiation grade. In contrast, CD56 was only rarely found in peripheral neuroepitheliomas. This might reflect differences in the cell-cell interactions between tumor cells in neuroblastoma and peripheral neuroepithelioma. Besides CD56 mAb, a series of mAb of the neuroblastoma and small cell lung cancer workshops detect molecules that share an epitope in common with N-CAM.¹⁹ These include, *e.g.*, mAb UJ13A and 3F8 binding of which has been demonstrated *in situ* in 4/4 neuroblastomas and *in vitro* in 6/8 neuroblastoma cell lines, respectively. Both UJ13A and 3F8 have gained diagnostic and/or therapeutic application.^{20,21} Since we found CD56 expressed in a great variety of normal peripheral nerves and autonomic ganglia, the diagnostic and therapeutic value of mAb recognizing N-CAM should be re-assessed. In addition to their expression in neuroblastomas, some of the mAb sharing an epitope in common with N-CAM including the CD56 group have also been demonstrated in medulloblastomas, Ewing's sarcomas, Wilms' tumors, and even in rhabdomyosarcomas.^{19,22} Furthermore, the long chain form of polysialic acid of N-CAM has been described in Wilms' tumors and in fetal kidneys by absence in adult ones.²³ Thus, N-CAM has been considered an onco-developmental antigen in renal tissue. Like other mAb sharing an epitope in common with N-CAM, CD56 was expressed in rhabdomyosarcomas.¹⁸ We also found CD56 expression in all rhabdomyosarcomas studied. In contrast to these data, none of our cases was completely CD56-negative. Additionally, and in parallel to the N-CAM expression in renal tissue, we found CD56 expression in fetal but not in adult skeletal muscles. Thus, we suggest that, in skeletal muscles, this antigen might also be considered an onco-development antigen. This suggestion is supported by the fact that N-CAM is re-expressed in regenerative muscle cells in various myopathies^{24,25} and, as shown in this study, in muscle fibers associated with tumor cells and in damaged muscle fibers, since regenerative muscle cells also follow the developmental pathway.

Taken together, the distribution pattern of CD56 in neural and skeletal muscle cells and their tumors generally corresponds to the known patterns of N-CAM expression. The absence of CD56 in some malignant schwannomas and in most peripheral neuroepitheliomas might lead to an abnormal cell migration potential. The pattern of CD56 expression in skeletal muscle cells and their tumors suggests

a role of this molecule as an onco-developmental antigen. These findings suggest parallels to the situation found in renal tissue. Against the background of the broad distribution pattern of CD56 in normal peripheral nerves and autonomic ganglia the diagnostic and therapeutic value of mAb sharing an epitope in common with N-CAM should be critically re-evaluated.

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